Biological control of mycotoxigenic fungi in cereals: A successful step to food safety

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Problem Statement

Over the last decade, several **BCAs** for management of ***Fusarium* diseases** such as Fusarium Head Blight (**FHB)** & Fusarium Ear Rot (**FER)** have been well documented in literature. Although, **FHB** and **FER** are caused by mycotoxigenic *Fusarium* species, the potential biocontrol effect on mycotoxins is **underexplored** (1). *Fusarium graminearum*, the main pathogen involved in **FHB & FER** in cereals, produces multiple mycotoxins such as DON and ZEN. The effect of BCAs on multiple mycotoxins as well as fungal growth has not been studied before.

Aim & Experimental Setup

- Screening for **novel** BCAs (focus on fungal endophytes).
- Test their ability to effectively suppress the fungal growth and infection (different *in vitro* and *in planta* assays for accurate selection of the best performing BCAs).
- Test their ability to effectively suppress the production of different mycotoxins (validated LC-MS/MS method for each matrix for toxins quantification).
- Identification of potential biocontrol molecules that exert or contribute into the biocontrol effect (HR-LCMS/MS).

1. Isolation of BCAs from Maize Stubble And Soil

Several maize stubble and soil samples

Isolation on
-PDA
-DG18 agar with 2.5 mg/l Malachite Green agar
-All media contained antibiotics

	Isolated strains	Abbreviation	Source
1	Clonostachys rosea (MFUG 1116, Belgium)	Cr4	Maize stubble
2	Epicoccum nigrum (MFUG 11701, Belgium)	En1	Soil
3	Epicoccum nigrum (MFUG 11702, Belgium)	En2	Soil
4	Epicoccum nigrum (MFUG 11703, Belgium)	En3	Soil
5	Bionectria ochroleuca (MFUG 011611, Belgium)	Bo1	Maize stubble
6	Bionectria ochroleuca (MFUG 021612, Belgium)	Bo2	Maize stubble
7	Sordaria fimicola (MFUG 1016, Belgium)	Sf1	Soil

	Reference strains	Abbreviation
1	Trichoderma harzianum (CBS 226.95, England)	Th1
2	Trichoderma harzianum (CBS 243.71, Switzerland)	Th2
3	Clonostachys rosea (CBS 100502, France)	Cr1
4	Clonostachys rosea (CBS 102.94, The Nederlands)	Cr2
5	Clonostachys rosea (CBS 100494, Australia)	Cr3
6	Sordaria fimicola (Roberge) Cesati & de Notaris (MUCL 29304, Argentina)	Sf2
7	Piriformospora indica DSM11827	Pi
8	Piriformospora williamsii (ex multinucleate rhizoctonia DAR29830)	Pw

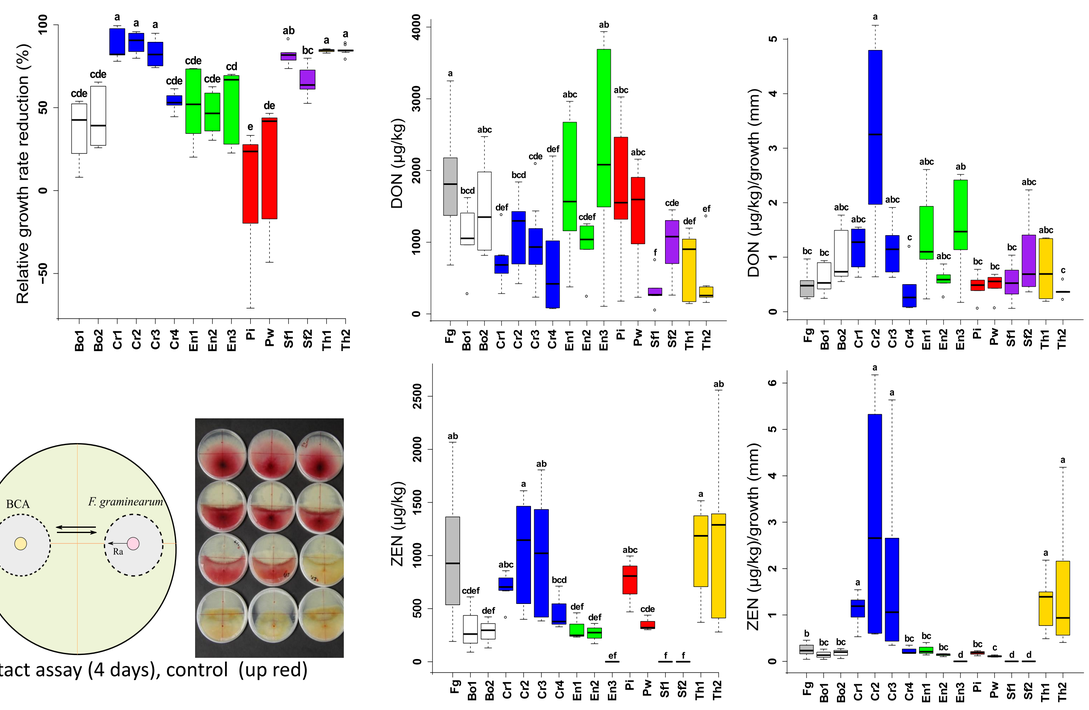
References strains from CBS (Nederlands), MUCL (Belgium) and University of Giessen (Germany)

Nucleotide BLAST

PCR

Gel electrophoresis

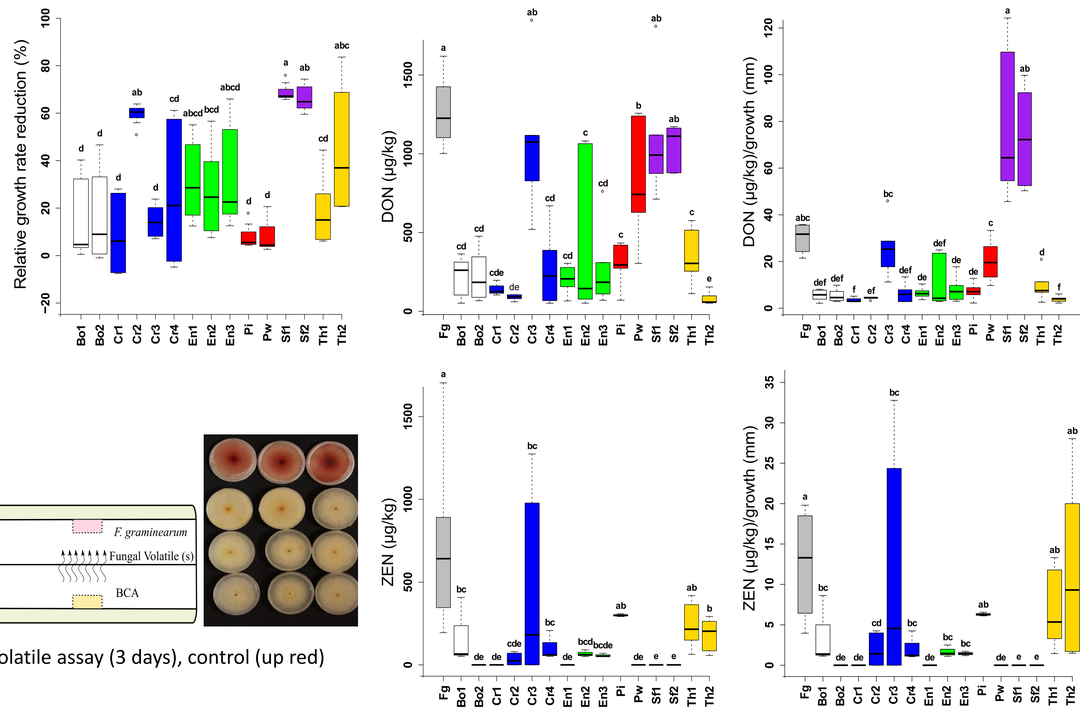
2. *In Vitro* Plating Assay



Contact assay (4 days), control (up red)

Conclusion:- The new isolated strains inhibit *Fusarium graminearum* growth. Mycotoxin reduction is attributed to reduction to fungal growth. The biocontrol effect is more clear in ZEN than in DON

3. *In Vitro* Volatile Assay

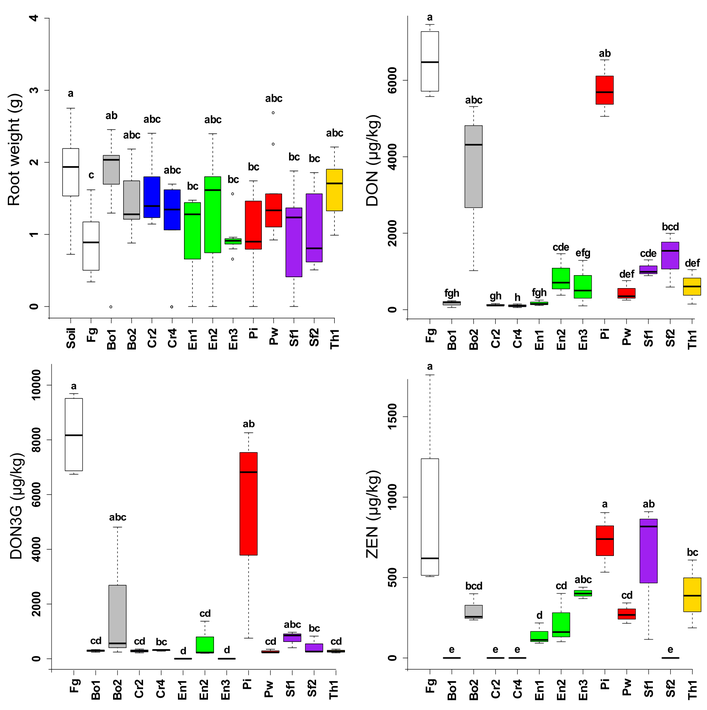


Volatile assay (3 days), control (up red)

Conclusion:- The volatile assay showed an inhibition of *Fusarium graminearum* growth which may be assumed due to the presence of some fungal volatile(s) or bioactive molecules produced by BCAs. Similar to plating assay, the biocontrol effect is more clear in ZEN than in DON. However, the effect on the mycotoxins exceeds the reduction in fungal growth which point to an active inhibition of mycotoxin production

4. *In Planta* (Maize) Assay

F. graminearum was cultured at 22 °C for 10 days on sterile rice. Each BCA was cultured in PDB at 25 °C for two weeks. Mycelium (12.5 gm ± 1) was mixed thoroughly with the soil. *F. graminearum* grown on rice was added to soil. Maize was grown for 2 weeks at RT, 12 h light/12 h darkness.



Maize pot experiments (2 weeks)

Conclusion:- The biocontrol effect is present for the majority of endophytic BCAs, although the effect on symptom development is minor and highly variable. The effect of the endophytic BCAs on mycotoxin production *in planta* is clear and more proliferated than the effect on the symptom development. *In planta*, the BCAs result in an active reduction of mycotoxin production by the pathogen.

Acknowledgements

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(1) Abdallah MF, Ameye M, Saeger S De, Audenaert K, Haesaert G. (2018) Biological control of mycotoxigenic fungi and their toxins: an update for the pre-harvest approach. In: Fungi and Mycotoxins - Their Occurrence, Impact on Health and the Economy as well as Pre- and Postharvest Management Strategies. IntechOpen (accepted)

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